

AMENDMENTS TO THE SPECIFICATION

Please amend paragraph [0047] on pages 41-42 of the specification as filed, as follows:

(Proteins encoding human shugoshin homologous gene are specifically localized at centromeres in mitosis)

The present inventor previously identified two putative human Sgo proteins, Sgo1 and Sgo2 in the database, although their overall sequence homology to known Sgo proteins in any species other than human is marginal (Fig. 11a). To examine whether these proteins identified in the database are actually human Sgo homologs, the present inventor examined the localization of the proteins. For this end, ~~the present inventor cultured rabbit polyclonal antibodies against recombinant proteins that were produced in bacteria~~ parts of hSgo1 and hSgo2 proteins (SEQ ID NOs: 18 and 20, respectively hSgo1 and hSgo2), encoded human shugoshin homologous gene (SEQ ID NOs: 17 and 19) that were presumed to be human Sgo homologues, were expressed in E. coli, and antibodies against hSgo1 and hSgo2 were produced. The obtained Sgo1 antibodies detected an up to 70kD band (predicted molecular weight is 60kD) in the HeLa cell extracts, and the signal was significantly reduced when cells were treated with siRNA that targets human Sgo1 mRNA (Fig. 11b). Similarly, Sgo2 antibodies detected an up to 120kD band (predicted molecular weight is 145kD), the signal was reduced in extracts obtained from cells treated with human Sgo2 siRNA (Fig. 11b). These data indicate that both Sgo1 and Sgo2 are expressed at least in proliferating HeLa cells. Next, ~~for the purpose of analyzing proteins (SEQ ID NOs: 18 and 20, respectively hSgo1 and hSgo2) encoding human shugoshin homologous~~

gene (SEQ ID NOs: 17 and 19) that was presumed to be human Sgo homologues, part of hSgo1 and hSgo2 was expressed in *E. coli*, and antibodies against hSgo1 and hSgo2 were produced by injecting the protein into rabbit. HeLa cells were stained with the antibodies against hSgo1 and hSgo2 and concurrently with tubulin antibodies and DAPI, and co-stained with spindle and chromosome DNA respectively, and the expression of hSgo1 and hSgo2 proteins that were both endogenous in proliferating cells was examined. The results are shown in figure 12. As shown in Figure 12, both signals of hSgo1 and hSgo2 were also observed as dots on chromosomes from prometaphase to metaphase. As a result of the immunostaining, it was identified that both proteins, hSgo1 and hSgo2 are specifically localized at centromeres at mitotic phase. Further, HeLa cells at prometaphase and metaphase were stained with antibodies against hSgo1 or hSgo2; concurrently co-stained with antibodies against centromere protein CENP-A, and DAPI; and examined the expression of hSgo1 and hSgo2 proteins. The results are shown in figure 13. As shown in Figure 13, both signals of hSgo1 and hSgo2 were observed at sites close to CENP-A dots on chromosomes. As a result of the above, it was revealed that both hSgo1 and hSgo2 are centromere proteins. Further, to examine this possibility, Aurora B, which is a passenger protein of chromosome known to be localized within kinetochore from prophase to metaphase, was stained. The sites of Sgo1 and Aurora B were practically the same at prometaphase and metaphase, whereas Sgo2 was placed just outside Aurora B (see Fig. 13). As a result of the above, it was revealed that both hSgo1 and hSgo2 are placed within kinetochores from prometaphase to metaphase. Representative views of sister kinetochore are magnified on the right. Scale bar is 10µm.